I. The Pending Claims Compy with 35 U.S.C. § 112, first paragraph

A. Rejection of claims 1-27, 29-33, 36, 38 and 39

Claims 1-27, 29-33, 36, 38 and 39 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate with the scope of the claimed invention. Applicants respectfully traverse this rejection.

The pending claims are directed to methods and compositions relating to the observation that T cells can be engineered ***ex vivo***, e.g., in culture outside of the body, with expression contructs encoding macrolide binding proteins (MBP) which have been mutated such that they have an altered binding affinity for a macrolide relative to the wild-type form of the MBP. When contacted with a macrolide which binds to the mutated MBP, activation of the engineered T cell can be inhibited. In certain embodiments, such as where one uses a macrolide which binds the mutated MBP with greater affinity than the wild MBP, the engineered T cells can be selectively inhibited when present in a mixed population including wild-type T cells. Thus, for example, the engineered T cells can be transplanted into an animal, and the macrolide (an organic molecule which can be administered by any of several routes to the animal) can be used to selectively inhibit activation of the engineered cells.

The previous office action, upon which the Examiner relies to support this rejection, argues that the instant invention is "complicated *in vivo* by the need to selectively transfect or transduce hematopoietic cells in a multicellular environment". The previous office action then goes on to reference articles directed to in vivo targeting of gene therapy.

However, the Examiner has explicitly acknowledged that the specification is enabling for "*in vitro* transfection of MBP to express mutated MBP". Office Action, Page 3 lines 9-10. The claims are limited to methods in which the T cells are or were engineered *ex vivo* - rather than *in vivo*. Accordingly, because the Examiner agrees that the specification enables the *ex vivo* engineering of T cells, the Examiner's argument concerning *in vivo* engineering are mute. By

The Examiner's remaining argument from the previous office action appears to be that the application has not sufficiently enabled the subsequent transplantation of those engineered cells into animals and treatment of the transplanted animal with a cognate macrolide for the mutated MBP. In particular, the Examiner has argued that there is a criticality to the level of expression of the mutated protein, e.g., cyclophilin or FKBP, that may be required for the successful inhibition of transcription or proliferation *in vivo*. However, Applicants note the presence of a variety of examples in the literature of successful expression of heterologous proteins in T cells leading to *in vivo* results. Applicants cited several articles in their last response showing in vivo biological activity of transduced T cells. In particular, Applicants submitted an article by Bonini et al. (1997) "HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia" *Science* 276: 1719 (1997). This article describes *ex vivo* transfection of peripheral blood mononuclear cells (PBMCs) with a viral vector encoding HSV thymidine kinase (TK), selection of the cells that were transfected (see Note 16), and infusion of these transfected cells to patients. The three patients who developed graft-versus-host disease received gancyclovir, and in these three patients, this led to a complete abrogation,

or to at least a decrease in the symptoms, of the graft-versus-host disease. As indicated, e.g., at page 1722, starting at line 8, "[b]ecause no immunosuppressive drug was administered to this patient, the complete abrogation of the severe liver GvHD could be attributed exclusively to the expression of the TK gene." Thus, in view of these results, a person of skill in the art would not doubt that a mutated immunophilin could be expressed in T cells to levels sufficiently high to induce inhibition of T cell proliferation or activation when contacted with a mutant ligand *in vivo*. There is no reason to believe that the level of expression of the immunophilin would have to be significantly different from the level of expression of the TK gene described in Bonini et al.

Thus, contrary to the Examiner's statements that "Bonini et al. profess that their data indicated only a possible increase in efficiency and safety of allo BMT," Bonini et al. clearly showed that the TK gene can be expressed *in vivo* to levels sufficient for the killing of the cells. Applicants believe that the Examiner based this statement on the last sentence of the abstract, and submit that it is routine in scientific publications to be cautious in expressing conclusions. Such a cautionary statement cannot, however, nullify the observations made by Bonini et al. which are described in the article. Accordingly, based on the literature, one skilled in this art would simply not expect any particular problem with achieving a sufficient heterologous expression level.

The law requires merely that there be a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity. Here, Applicants have provided working examples showing that mutated immunophilins expressed in cells bind to mutated ligands and are biologically active. Based on these teachings, as well as the numerous scientific publications describing expression of proteins in T cells *in vivo* (e.g., TK gene described in Bonini et al.), a person of skill in the art would reasonably anticipate expression of the mutated immunophilins to lead to the claimed biological activity *in vivo*. Thus, since the relevant evidence as a whole shows the existence of a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity, reconsideration of this rejection is respectfully requested.

It is respectfully submitted that maintenance of the present rejection is not supported by substantial evidence, and as such, does not meet the "arbitrary, capricious" standard applied under the "substantial evidence" test of Section 706(2)(E) of the Administrative Procedure Act. No relevant or other fact finding has been cited or relied upon by the Examiner to rebut the Applicants' arguments. Dickinson v. Zurko, 119 S. Ct. 1816 (1999).

B. Rejection of claims 40-43

Claims 40-43 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to "reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention". In particular, the Examiner argues

> [A]ll analogs of cyclosporin, rapamycin or FK506 have not been described in the specification. In the absence of such, it is not clear what specific analogs applicants are claiming...

Applicants respectfully traverse this rejection.

The present specification makes clear that the choice of mutated MBP and macrolide is to provide a system in which the chosen macrolide is more selective (not less, as the Examiner states) for the mutated MBP than for the wild-type protein. Thus, the macrolide will be more

potent, relative to treating wild-type T cells, at inhibiting activation of T cells which have been engineered to express the mutated MBP. Thus, in a mixed population of engineered and wild-type T cells, the activation of the engineered T-cells can be selectively inhibited.

For purposes of clarity of Applicants' arguments, Applicants summarize in this paragraph the knowledge in the art of immunosupressants and their mechanism of action at the time the application was filed. It was known at the time that cyclosporin and FK506 are among the most potent immunosuppressive drugs. It was also well known in the art that their potent immunosupressive action is primarily mediated by inhibition of activation and proliferation of T cells (see, e.g., Schreiber and Crabtree (1992) "The mechanism of action of cyclosporin A and FK506" *Immunology Today* 13: 136-42 (previously made of record), e.g., at line 4 of the left column of page 136 and at lines 3-6 of the left column of page 137). It was further well known in the art that this effect is primarily mediated by the binding of cyclosporin and FK506 to their corresponding immunophilins, i.e., cyclophilin and FKBP, respectively, thereby forming a molecular complex. The immunosuppressive effect of these drugs is mediated by the binding of this molecular complex to calcineurin to form a ternary complex, thereby inactivating calcineurin. Thus, calcineurin was known to be is a critical element in immunosuppression and in inhibiting T cell activation (see, e.g., page 38, lines 13-21 of the specification and Clipstone et al. (1992) "Identification of calcineurin as a key signaling enzyme in T-lymphocyte activation" *Nature* 357: 695, previously made of record, and page 138 of Bierer B.E. (1994) *Chem. Immunol.* 59: 128, previously made of record). It was also well known in the art, that one of the biological activities of calcineurin regulates NF-AT translocation to the nucleus and thereby regulates the transcriptional activation of NF-AT dependent genes, e.g., IL-2 and other cytokine genes, which are involved in T cell activation (see, e.g., Clipstone and Crabtree (1993) "Calcineurin is a key signaling enzyme in T lymphocyte activation and the target of the immunosuppressive drugs cyclosporin A and FK506" *Ann. NY Acad. Sci.* 696:20, at lines 4-7 of the left column of page 20, previously made of record). In particular, calcineurin enables NF-AT to translocate to the nucleus and activate gene transcription.

Thus, blocking NF-AT dependent gene transcription would result at least in part in inhibition of T cell activation. Furthermore, in view of the key role of calcineurin in regulating NF-AT, the observation that NF-AT dependent gene transcription is inhibited by a modified form of a compound which in its native form inhibits calcineurin indicates that the modified compound also inhibits calcineurin. Since calcineurin is a key player in T cell activation and immunosuppression, inhibition of calcineurin by the modified compound would also result in inhibition of T cell activation and establishment of immunosuppression.

The specification provides practical examples showing that incubation of T cells, which were transfected with a construct encoding a mutated cyclophilin, with a mutated cyclosporin, which binds selectively to the mutated cyclophilin relative to the wild-type cyclophilin, induces a biological response in the cell, as evidenced by the inhibition of transcription of a gene responsive to a signal transduction pathway that is blocked by cyclosporin. Thus, the working example provided in the specification demonstrates that native proteins, e.g., cyclophilins and FKBPs, and their corresponding native ligands can each be mutated in such a way that they bind selectively to each other, i.e., that they essentially do not interact with the native macrolide-binding protein and the native ligand, respectively, and yet retain their biological activity.

Applicants respectfully submit that the specification teaches how to make and use the invention commensurate with the scope of the claimed invention. The specification describes

mutated proteins, such as cyclophilin and FK506 binding proteins, which bind to mutated ligands, in particular, mutated cyclosporin and mutated FK506, respectively. The specification teaches how to identify other mutated ligand-binding proteins and ligands binding thereto (see, e.g., pages 18-21). For example, the specification teaches that modified two-hybrid assays can be used for that purpose.

The specification also provides practical examples showing that incubation of T cells, which were transfected with a construct encoding a mutated cyclophilin, with a mutated cyclosporin, which binds selectively to the mutated cyclophilin relative to the wild-type cyclophilin, induces a biological response in the cell, as evidenced by the inhibition of transcription of a gene responsive to a signal transduction pathway that is blocked by cyclosporin. Thus, the working example provided in the specification demonstrates that native proteins, e.g., cyclophilins and FKBPs, and their corresponding native ligands can each be mutated in such a way that they bind selectively to each other, i.e., that they essentially do not interact with the native macrolide-binding protein and the native ligand, respectively, and yet retain their biological activity.

Regarding FRAP and calcineurin, Applicants respectfully submit that the specification teaches mutations in FRAP and corresponding mutations in rapamycin (see, e.g., page 18). The specification further teaches how other mutations can be made in FRAP or calcineurin, or other macrolide-binding proteins, and how to confirm that such mutated macrolides are active in inhibiting T cell activation of T cells expressing a mutated macrolide binding protein.

Based on the teachings of the specification and the general knowledge in the art at the time the invention was made, a person of skill in the art could reasonably infer that it is possible to mutated FRAP, calcineurin and other MBPs, such that they retain their biological activity, e.g., inhibiting T cell activation, without undue experimentation.

Thus, relying on the working examples of the specification and the general knowledge in the art at the time the application was filed, a person of skill in the art could reasonably have anticipated at the time the application was filed, that a modified form of a native compound, e.g., cyclosporin and FK506, which native compound inhibits T cell activation by inhibiting calcineurin and NF-AT dependent gene transcription, also inhibits calcineurin and T cell activation if the modified form of the native compound inhibits NF-AT dependent gene transcription.

In view of all of the above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 112, first paragraph.

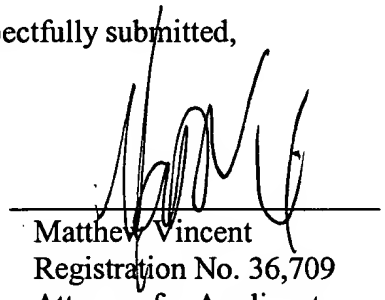
II. The Claimed Subject Matter is Patentable over the Art

Applicants note with appreciation that the claims were found to be free of the art.

III. Conclusion

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 951-7000.

Respectfully submitted,



Matthew Vincent
Registration No. 36,709
Attorney for Applicants

Patent Group
ROPES & GRAY
One International Place
Boston, MA 02109
Tel (617) 951-7000
Fax (617-951-7050

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. **(Twice Amended)** A method for inhibiting activation of a T cell, wherein the T cell or a progenitor cell thereof was engineered *ex vivo* to express a gene encoding a mutated macrolide binding protein (MBP), which method comprises contacting the cell with a macrolide which induces macrolide-dependent inhibition of activation of the T cell in a manner dependent on the expression of the mutated MBP.

2. **(Twice Amended)** A method for inhibiting transcription of an NF-AT dependent gene in a T cell, wherein the T cell or a progenitor cell thereof was engineered *ex vivo* to express an MBP gene encoding a mutated macrolide binding protein (MBP) which method comprises contacting the T cell with a macrolide which selectively binds to the altered MBP relative to the wild-type MBP and induces macrolide-dependent inhibition of transcription of NF-AT dependent gene in the T cell in a manner dependent on the expression of the mutated MBP.

3. **(Reiterated)** The method of claim 1 or 2, wherein the MBP is selected from the group consisting of a FRAP, an FK506-binding protein, a cyclophilin and a calcineurin.

4. **(Reiterated)** The method of claim 1 or 2, wherein the macrolide binds the mutated MBP with a dissociation constant, Kd, at least one order of magnitude less than its Kd for binding to wild-type MBP.

5. **(Reiterated)** The method of claim 4, wherein the macrolide binds the mutated MBP with a dissociation constant, Kd, at least three orders of magnitude less than its Kd for binding to wild-type MBP.

6. **(Twice Amended)** The method of claim 1 or 2, wherein the MBP gene was introduced into the cell *ex vivo* by DNA transfection.

7. **(Twice Amended)** The method of claim 1 or 2, wherein the MBP gene was introduced into the cell *ex vivo* by virus-mediated transduction.

8. **(Twice Amended)** The method of claim 1 or 2, wherein the MBP gene was introduced into the cell *ex vivo* by homologous recombination.

9. **(Reiterated)** The method of claim 1 or 2, wherein the macrolide is an analog of rapamycin, FK506 or cyclosporin.

10. **(Reiterated)** The method of claim 1 or 2, wherein the MBP gene encodes a FRAP protein, and the macrolide is an analog of rapamycin.

11. **(Reiterated)** The method of claim 1 or 2, wherein the MBP gene encodes an FK506 binding protein, and the macrolide is an analog of FK506 or rapamycin.

12. **(Reiterated)** The method of claim 1 or 2, wherein the MBP gene encodes a calcineurin protein, and the macrolide is an analog of FK506 or cyclosporin.

13. **(Reiterated)** The method of claim 1 or 2, wherein the MBP gene encodes a cyclophilin protein, and the macrolide is an analog of cyclosporin.

14. **(Reiterated)** The method of claim 1 or 2, wherein the cell is a mammalian cell.

15. **(Reiterated)** The method of claim 1 or 2, wherein the cell is a human cell.

16. **(Twice Amended)** A method for selectively inhibiting T cell activation in a transplanted T cell comprising

(i) transplanting, into an animal, a T cell or a progenitor cell thereof, which T cell or progenitor cell thereof which has been engineered *ex vivo* to express an MBP gene encoding a mutated macrolide binding protein (MBP), the mutated MBP having an altered macrolide-binding specificity relative to the wild-type form MBP_s and

(ii) administering to the animal an amount of a macrolide sufficient to inhibit activation of the transplanted T cell or progenitor cell thereof, which macrolide selectively induces macrolide-dependent inhibition of activation of the transplanted T cell, in a manner dependent on the expression of ~~expressing~~ the mutated MBP, when compared to cells expressing a wild-type form of the MBP.

17. **(Reiterated)** The method of claim 16, wherein the MBP is selected from the group consisting of a FRAP, an FK506-binding protein, a cyclophilin and a calcineurin.

18. **(Reiterated)** The method of claim 16, wherein the macrolide binds the mutated MBP with a dissociation constant, Kd, at least one order of magnitude less than its Kd for binding to wild-type MBP.

19. **(Reiterated)** The method of claim 16, wherein the macrolide binds the mutated MBP with a dissociation constant, Kd, at least three orders of magnitude less than its Kd for binding to wild-type MBP.

20. **(Twice Amended)** The method of claim 16, wherein the MBP gene was introduced into the cell *ex vivo* by DNA transfection.

21. **(Twice Amended)** The method of claim 16, wherein the MBP gene was introduced into the cell *ex vivo* by virus-mediated transduction.

22. **(Twice Amended)** The method of claim 16, wherein the MBP gene was introduced into the cell *ex vivo* by homologous recombination.

23. **(Reiterated)** The method of claim 16, wherein the macrolide is an analog of rapamycin, FK506 or cyclosporin.

24. **(Reiterated)** The method of claim 16, wherein the animal is a mammal.

25. **(Reiterated)** The method of claim 24, wherein the animal is a human.

26. **(Reiterated)** The method of claim 16, wherein the transplanted T cell is autologous to the animal.

27. **(Reiterated)** The method of claim 16 or 26, wherein the transplanted T cell is present within transplanted bone marrow.

29. **(Reiterated)** The method of claim 16, wherein the expression of the mutated MBP gene is transcriptionally regulated by a T-cell specific transcriptional regulatory sequence.

30. **(Reiterated)** The method of claim 16, wherein the animal is in an immunosuppressed state.

31. **(Reiterated)** A method for reducing graft-versus-host disease in an animal by selectively inhibiting T cell activation of a transplanted T cell, comprising

(i) prior to transplanting a T cell or a progenitor cell thereof, *ex vivo* engineering the T cell or progenitor cell thereof with a gene encoding a mutated macrolide binding protein (MBP), which is a mutated form of a native protein selected from the group consisting of FKBP and cyclophilin, the mutated MBP having an altered macrolide-binding specificity relative to the wild-type form MBP; and

(ii) subsequent to transplanting the T cell or a progenitor cell thereof, administering to the animal an amount of a macrolide sufficient to inhibit activation of the transplanted T cell or progenitor cell thereof, which macrolide selectively induces macrolide-dependent inhibition of activation of the transplanted T cell expressing the mutated MBP compared to endogenous cells of the animal, such that graft-versus-host disease is reduced.

32. **(Reiterated)** An expression construct encoding a mutated FRAP, FKBP, cyclophilin or calcineurin, wherein the mutated protein has an altered macrolide-binding specificity relative to its wild-type form and, in the presence of a macrolide to which it binds, induces macrolide-dependent inhibition of activation of a T cell expressing the mutated protein.

33. **(Reiterated)** A kit for selectively inhibiting activation of a T cell, comprising

(i) an expression construct of claim 32 and

(ii) a macrolide which selectively binds to the altered protein relative to the wild-type protein and selectively induces macrolide-dependent inhibition of activation of T cells expressing the mutated MBP relative to T cells expressing only the wild-type MBP.

36. **(Reiterated)** An isolated population of cells comprising a T cell or progenitor cell thereof, which is transfected with an expression construct of claim 32.

38. **(Reiterated)** A method for rendering a T cell susceptible to inhibition of activation by a macrolide, comprising transfecting a T cell *ex vivo* with a nucleic acid encoding MBP to which the macrolide binds selectively relative to the unmodified MBP, which modified MBP retains the ability to cause macrolide-dependent inhibition of activation of the T cell.

39. **(Reiterated)** The method of claim 38, comprising the further step of introducing the transfected T cell into a recipient mammal.

40. **(Reiterated)** The method of claim 16, 31, or 38, wherein the mutated MBP is a mutated form of an FK506 binding protein, and the macrolide is an analog of FK506 or rapamycin.

41. **(Reiterated)** The method of claim 16, 31, or 38, wherein the mutated MBP is a mutated form of a cyclophilin protein, and the macrolide is an analog of cyclosporin.

42. **(Reiterated)** The kit of claim 33, wherein the mutated MBP is a mutated form of an FK506 binding protein, and the macrolide is an analog of FK506.

43. **(Reiterated)** The kit of claim 33, wherein the mutated MBP is a mutated form of a cyclophilin, and the macrolide is an analog of cyclosporin.

44. **(Reiterated)** The expression construct of claim 32, which encodes a mutated FKBP or cyclophilin.